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AEROBIOLOGY, ALLERGENICITY AND BIOCHEMISTRY OF *MADHUCA INDICA* GMEL. POLLEN

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Abstract: An ASTIR volumetric sampler was used for one year (May 1995–April 1996) for aerobiological survey at Beharampore town, a centrally located representative part of West Bengal, to record the occurrence and frequency of airborne *Madhuca* pollen. The highest frequency of *Madhuca* pollen was recorded in April when the weather was dry with low relative humidity (RH) and moderately high temperature. Clinical test (skin prick test) showed *Madhuca* pollen to be one of the major causes of respiratory allergy. 30–60% $(NH_4)_2SO_4$ cut fraction showed maximum positivity in skin prick test. Biochemical analysis showed that *Madhuca* pollen was rich in lipid and protein. SDS-PAGE was performed with the total soluble pollen protein which showed a total of 6 major protein bands, while in isolated fraction (Fr. II) a total of 7 protein bands were obtained.

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Key words: Pollen, *Madhuca indica*, aerobiology, allergy, biochemistry, skin prick test, SDS-PAGE.

INTRODUCTION

Madhuca indica Gmel. (family *Sapotaceae*) or 'Mahua' is a common tropical tree in jungles and rural areas of India. This plant is economically important because of the role it plays in yielding country liquor, edible succulent corollas and oil from the seeds. *Madhuca* pollen grains are found in air and have been proved to be allergenically potent.

It is reported that about 10% of the Indian population suffer from different types of allergic diseases and the majority of these are from pollen allergy [8]. Thus, from the clinical point of view it is important to determine the details about the occurrence of allergenic pollen grains in the atmosphere, their chemical composition and molecular characterization. In the present paper an attempt has been made to determine the seasonal distribution of *Madhuca* pollen in air with reference to their allergenic potency as evidenced by the results of skin test and chemical analysis.

MATERIALS AND METHODS

The incidence of *Madhuca* pollen grains was carried out using an ASTIR one day volumetric sampler (suction rate 10 l/min). The sampler was placed about 4 m above ground level in a domestic house in Beharampore town. Meteorological data of Beharampore town were collected from the Pulses and Oil Seeds Research Centre, Beharampore, West Bengal.

To test allergenicity, allergenic extracts were prepared from bulk uncontaminated pollen grains following the method of Sheldon *et al.* [6], modified considering the total conditions by Shivpuri [7] and Gupta and Chanda [2]. Firstly, the dried pollen grains were defatted with solvent ether and then extracted in phosphate buffer (pH 7.5) and diluted in 1:50 ratio for skin test. Before the skin test, the sterility of antigenic extract was confirmed. The skin prick tests were performed at the Allergy Unit of the

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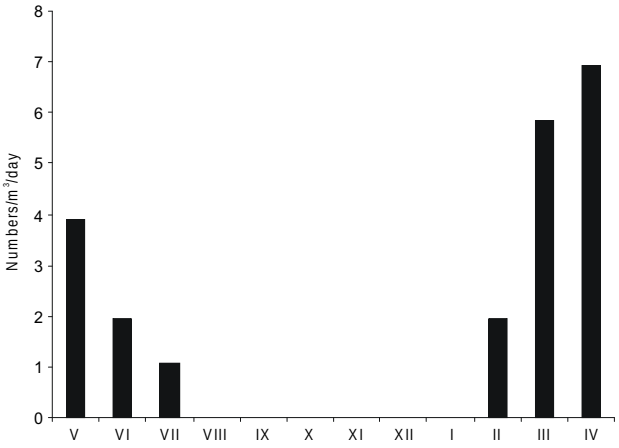

Figure 1. Average monthly concentration of *Madhuca indica* pollen.

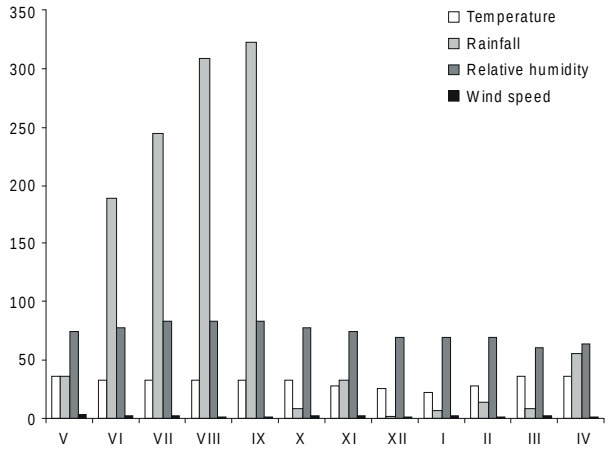

Figure 2. Meteorological parameters (1995-96) of Beharampore town. Temperature is expressed in degrees Celsius (ºC), rainfall in millimeters (mm), relative humidity in percent (%), wind speed in kilometers per hour (km/h).

Table 1. Correlation between meteorological factors and monthly total pollen count of *Madhuca indica*, expressed as coefficient of correlation (r) values.

	Temp.	RF	RH	WS
RF	0.249			
RH	0.118	0.828		
WS	0.452	0.247	0.382	
TP	0.654	-0.289	-0.598	-0.037

Degrees of freedom (DF) = 10; Temp. - Temperature, RF- Rainfall, RH- Relative humidity, WS - Wind speed, TP- Total Pollen

Table 2. Results of skin test with *Madhuca indica* pollen antigen.

Total number of patients	Number of positive reactions (%)	Intensity of reactions (%)		
		1+	2+	3+
109	22.93	20.18	2.75	0

Table 3. Results of skin test using fractions of *Madhuca indica* pollen antigen.

$(NH_4)_2SO_4$ cut fractions	Number of positive reactions (%)
Fr.I (0-30%)	18.75
Fr.II (30-60%)	58.75
Fr.III (60-90%)	50.00

Table 4. Chemical analysis of the pollen of *Madhuca indica* (percent of dry weight).

Total soluble carbohydrate	Total protein	Total lipid
2.7	7.7	24

Institute of Child Health, Calcutta, on adult respiratory allergic patients. A control test was performed with sterile buffer saline. The results were analysed according to Stytis *et al.* [10].

The whole allergenic extract was then fractionated in the range of 0–30%, 30–60% and 60–90% saturation of $(NH_4)_2SO_4$ cut and centrifuged. Each precipitated fraction was dissolved in PBS and separately dialyzed to remove the traces of ammonia. These fractions were referred to as Fr. I, Fr. II and Fr. III respectively. The allergenic activities of the fractions were determined by skin prick test. Before skin testing, the patients were advised to stop using antihistamine drugs and steroids for 72 hours. A detailed description of the symptoms, the case history, and if necessary, the examination of urine, blood, stool, nasal smear, throat scrab culture, chest radiogram, etc. were taken into account.

The total soluble carbohydrate content was estimated according to the method of Duboise *et al.* [1]. Total soluble protein was estimated following the method of Lowry *et al*. [5], using bovine serum albumin standard curve. Total lipid was estimated using the method of Itoh and Kaneko [3].

Finally, 11% SDS-polyacrylamide gel electrophoresis was performed for total soluble protein according to the method of Laemmli [4]. The extraction of soluble protein was made in sample buffer (0.5 M Tris HCl, pH 6.8, 2% SDS, 10% glycerol, 5% β-mercaptoethanol, 0.1% bromophenol blue dye) with heating for 2½ minutes at 100ºC before loading. The amount of protein was 40 µg per channel and the total current was 14 mA. The gel run was carried out in Tris-glycine buffer (pH 8.3) with 0.1% SDS. The gel was calibrated with a marker protein obtained from Genei private LTD., Bangalore, India. The gel was covered within a plastic bag and scanned on DE 300 densitometer (Biomidi, France). 11% SDS – PAGE was also carried out in the case of the most active $(NH_4)_2SO_4$ cut fraction (Fr. II) of *Madhuca* pollen.

RESULTS AND DISCUSSION

The presence of *Madhuca* pollen in air was recorded from the last week of February to June (Fig. 1), with a maximum frequency in April. The count dropped to zero or a very low level in July–August, probably due to heavy rainfall, high relative humidity and moderate high wind speed (Fig. 2). From September to January no *Madhuca* pollen were recovered from air. It is evident that *Madhuca* pollen dispersal was initiated by dry weather with low relative humidities (50–60%) and relatively high temperature (38ºC), because in such conditions they become light and dry and can be disseminated in the air with less constraint. In high humidities pollen may become heavy, which prevents them from remaining suspended in air as easily as in dry condition. Thus, the pollen concentration depends upon the climatic factors and this has been statistically supplemented (Tab. 1). A correlation was found between the monthly total pollen counts (Fig. 1) and meteorological factors (Fig. 2) using statistical analysis with aid of MINITAB computer programme. From the values of correlation coefficient (r) (Tab. 1) it was found that the pollen concentration was positively correlated with temperature and negatively correlated with rainfall and relative humidity. The level of significance (p-value) in the case of temperature and relative humidity was between 0.05–0.01, while it was below 0.1 for rainfall and wind speed. It was evident that the frequency of incidence of pollen was high in low relative humidity, medium temperature and minimum rainfall.

Skin prick test was performed with the whole pollen extract on 109 patients having relevant case history. As much as 23% of the patients showed positive response exhibiting 1+ and 2+ reaction level (Tab. 2). When skin test was performed with the three fractions (i.e., Fr. I, Fr. II and Fr. III) on 16 *Madhuca* sensitive patients, Fr. II elicited maximum percentage (58.75%) of positive allergic reactions compared to Fr. I (18.75%) and Fr. III (50%) (Tab. 3).

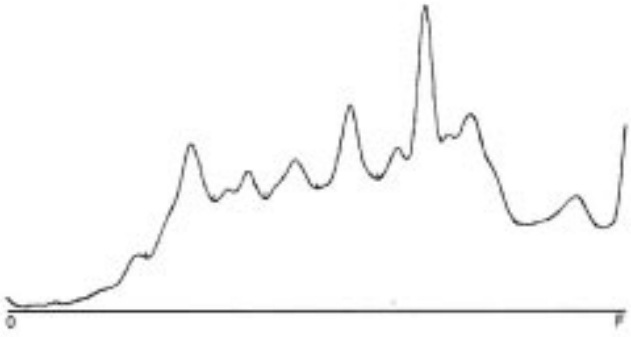

Figure 4. Densitograph profile of soluble proteins of pollen grains of *Madhuca indica.* Migration is from anode to cathode. O: Origin, F: Front.

The preliminary chemical studies (Tab. 4) on this pollen type revealed that the percentage of lipid was highest (24%) with a moderate amount of soluble protein (7.7%) and a low level of soluble carbohydrate (2.7%). Stanley and Linskens [9] reported that the protein level in pollen generally remains between 5.9–28.3% of pollen residue. Thus, the protein level of *Madhuca* pollen is

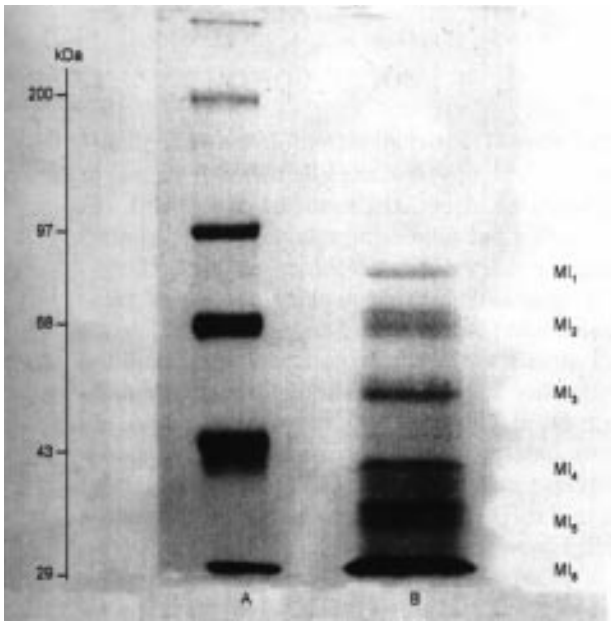

Figure 3. 11% SDS-PAGE of total soluble protein of *Madhuca indica* pollen. A. Molecular weight marker. B. Extract of *Madhuca indica* pollen protein.

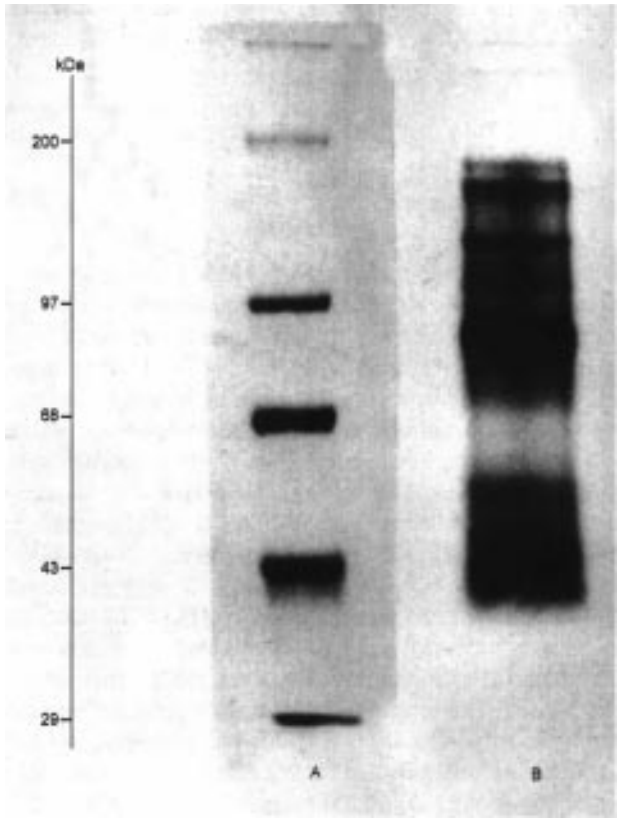

Figure 5. 11% SDS-PAGE of most active allergenic fraction (Fr. II) of *Madhuca indica* pollen. A. Molecular weight marker; B. Most active fraction of *Madhuca indica*.

found to be comparatively low. A high amount of lipid is utilized during germination as a food for growth and development.

11% SDS-PAGE of soluble protein of *Madhuca* revealed 6 major bands between the molecular weight range of 29–97 kDa (Fig. 3). These bands were designated as MI_1 (75 kDa), MI_2 (68 kDa), MI_3 (50 kDa), MI_4 (40 kDa), MI_5 (35 kDa) and MI_6 (29 kDa). Some faint bands were also observed. The densitograph profile of soluble proteins of this pollen type also showed 6 major peaks (Fig. 4). 11% SDS-PAGE of most active fraction (Fr. II) revealed a total of 7 protein bands showing a range of molecular weight between 43–200 kDa (Fig. 5).

Results of our study confirm that *Madhuca* is a common tropical airborne pollen in India between February and June with a peak concentration in April. It was found that these pollen grains are allergenically potent.

CONCLUSIONS

1. Volumetric air survey by ASTIR one day sampler revealed that the *Madhuca* pollen was a common component of the air of Beharampore town of West Bengal showing its maximum frequency in dry weather conditions when the rainfall and humidity was low.

2. It was found by skin test that *Madhuca* pollen is one of the common aeroallergens causing 23% positive reactions among respiratory allergic patients.

3. A total of 6 protein bands were obtained from *Madhuca* pollen within a molecular range of 29–97 kDa.

4. Among the $(NH_4)_2SO_4$ cut fractions, fraction II (30–60%) showed highest allergenicity (58.75% of positive skin reactions) with 7 major protein components between the molecular weight range of 43–200 kDa.

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